Spongins: nanostructural investigations and development of biomimetic material model

Hermann Ehrlich, Manuel Maldonado[1], Thomas Hanke, Heike Meissner[2], René Born, Dieter Scharnweber, Hartmut Worch

Max-Bergmann-Center of Biomaterials, Institute of Material Science, Dresden University of Technology, D-01062 Dresden, Germany

[1] Centro de Estudios Avanzados de Blanes (CSIC), E-17300, Blanes, Girona, Spain

[2] University Hospital „Carl-Gustav Carus“, D-01307 Dresden, Germany

Natural structural biomaterials provide an abundant source of novel bone and cartilage replacements and initiate the investigations for development of nano-sized biomimetic composites.

Sponges (Porifera) are presently gaining increased scientific attention because of their secondary metabolites and specific skeleton structures. Unique and innovative structural leads are discovered with cytotoxic, antifouling, antitumoral, antibiotic, antiviral or cyto-protective, enzyme-inhibitory, anti-inflammatory and anti-Alzheimer activities /1/. Sponge collagen easily forms nanoparticles useful as drug carrier systems /2/. Crookewitt first pointed out in 1843 that the endoskeleton of the common bath sponge is derived from the dermal (horny) layer and called him spongin /3/. Till now spongin (named also fibrous skeleton, pseudokeratin, neurokeratin, horny protein, collagen-like protein, scleroprotein) has no clear chemical definition.

From biomaterial point of view spongin-containing fibrous skeletons and spongial multilayered skeleton structures must be important also as materials for biomedical applications. The practical value of these skeletons is due to their large internal surface area estimated at between 25 and 34 m^2 for a 3- to 4-gram skeleton, which enables considerable liquid absorption to take place by capillary attraction /4/. Spongin based fibres and filaments were resistant to bacterial collagenases, pepsin, trypsin, chymotrypsin, pronase, papain, elastase, lysozyme, cellulase and amylase /5/. Weak acid or alkaline hydrolysis were no more effective. The fibres withstand treatment with 5% trichloracetic acid at 90°C /4, 5/. In spite of this behaviour the composition of the cuticular layers of keratose sponges fibres is unknown. Likewise, the potential role of spongins in formation of functional natural bio-composites is virtually unexplored. Less information is available about how spongin fibers are secreted by spongocytes. The role of spongin in Porifera biomineralization phenomenon is poorly understood. The purpose of the present study was to obtain understanding of the

nanotopography of spongin fibres surfaces, the nature of multilayered structures and nanoscale mineralized textures, the nanolocalization of biologically active bromotyrosine derivatives, the biomimetic potential of spongin based formations for the future development of new biologically inspired materials for biomedical applications.

Materials and methods.

The material studied consists of three keratose sponges specimens: Verongula gigantea, Aiolochroia crassa, Spongia agaricina. The ultra-structural morphology of the sponges surfaces and fibres was characterized by means of ESEM XL30, Phillips instrument. Energy dispersive X-ray microanalysis (EDX) was carried out using the same instrument. Samples were sputter-coated with a thin layer of gold (Sputtercoater S150B, Edwards). AFM-images of samples were obtained on Bioscope (DI/Veeco), tapping mode on air, cantilever with super sharp-tip (Nanosensors-SSS). FTIR spectra were obtained using a Perkin Elmer spectrometer FTS2000. Raman spectra were measured on a FT-Raman spectrometer RFS 100/S, Bruckner. The fluorescence spectra were recorded on a confocal laser scanning microscope (Zeiss LSM 510 META). Demineralization of samples was carried out using
2.5 N NaOH-extraction-procedure for keratin-containing structures /6/.
(+)-Aeroplysinin-1 from Aplysina aerophoba, Calbiochem, was used as a marker for identification of bromotyrosine derivatives.

Results and Discussion

The SEM micrographs presented in Figs. 1, 2, 3 show the surfaces and cross sections of V. gigantea, A. crassa and S. agaricina fibers, respectively. The fibers appears to be multilayered. At a higher magnification Fig. 4 shows a typical view of spongal fibers ultrastructure. The fibers of V. gigantea and A. crassa consists of three layers (Figs. 4, 5): a cuticule-like 5,50-6,50 μm thick outermost layer, a crystalline-like 60-70 μm thick cylinder forming the bulk of the fibre and an axial 10-15 μm thick thread in the center (Fig. 5). No axial thread, but middle channel was found within fibers of S. agaricina (Fig. 3).

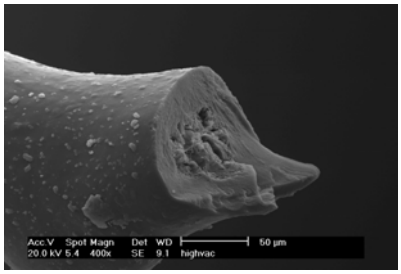

Fig. 1: Cross section of V. gigantea fibre

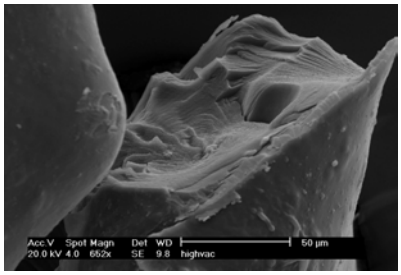

Fig. 2: Surface of A. crassa fibre

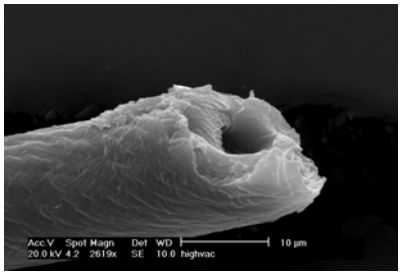

Fig.3: SEM image of the hollow fibre of S. agaricina

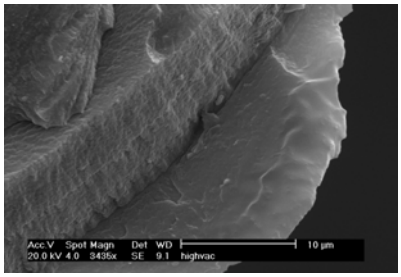

Fig. 4: Multilayered ultrastructure of A. crassa fibre

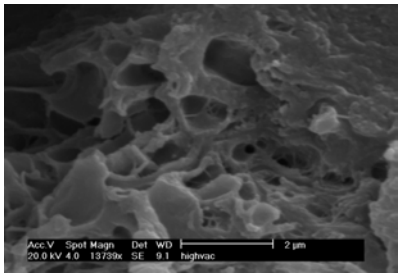

Fig. 5: Collagen-like axial thread in the middle of A. crassa fibre

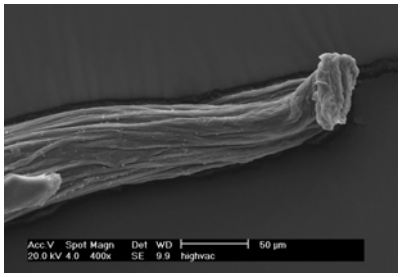

Fig. 6: V. gigantea fibre after alkali treatment

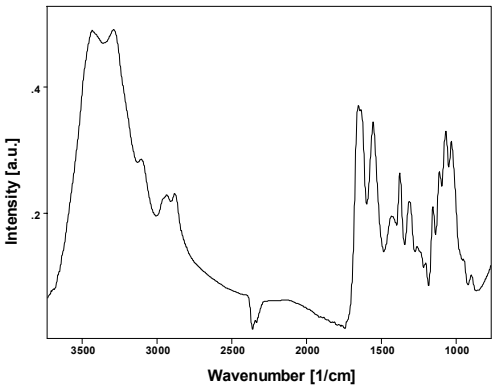

Fig. 7: IR spectra of V. gigantea and A. crassa chitin envelopes

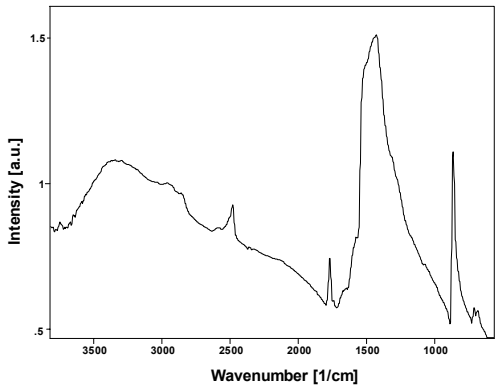

Fig. 8: IR spectra of aragonit-containing layer revealed in V. gigantea, A. crassa and S. agaricina fibers

Elemental analysis (EDX/ESEM) of V. gigantea fibre schows differences in the elemental composition between outher layer (C-80.06; O-18.67; Br-0.45; S-0.21; Cl-0.37; Ca-0.04; I-0.11 (At%)) and axial cylinder (C-83.21; O-11.6; Br-0.84; S-1.36; Cl-0.95; Ca-0.29; I-0.15 (At%)). The presence of Br and I in Verongiida sponges was also suggested previously by Block and Bolling /3/. Demineralization procedure by 2.5 N NaOH treatment was used to obtain the understanding of the nature and nanostructure of the spongal mineral component. On the 10th day of demineralization we observed only the presence of colourless soft hose-like envelopes (Fig.6). Results of FTIR analysis (Fig.7) give strong evidence to IR-spectra obtained by Brugnerotto et al. /7/ for chitin. The identification of crystalline-like layer and axial thread of V.gigantea and A.crassa by means of Raman (data not shown) and FTIR spectroscopy (Fig.8) shows aragonite and collagen-like fiber protein (AFM, data not shown) behaviour, respectively. No chitin or collagen-like proteins, but aragonite, were identified within fibers of S.agaricina. Si-containing spicules (EDX/ESEM identification) were also investigated within the proteinacous matrix only of this sponge.

Results of bromotyrosine nanodistribution investigations using LSM and natural aeroplysinin as marker clearly indicate the presence of bromotyrosine derivatives in all structural layers of

V. gigantea and A. crassa fibers, including chitinous cuticula after NaOH treatment (data not shown). No aeroplisinin-like compounds were identified by S. agaricina. Material properties of A. crassa fibrous matrix were measured as compression stress (0,5 N/mm^2) and compressive strain (>200 %). Obtained results correlate good with known properties of high resistance elastic materials.

Thus, the results of present study reveal for the first time direct evidence that chitin, aragonite, collagen-like fiber proteins and bromotyrosines are an important compounds of the fibers in investigated samples of V. gigantea and A. crassa. A comprehensive understanding of these fibers with respect to chemical composition, structure, and mineralization behaviour may prove to be an novel model for biomimetic synthesis of "chitin-mineral-bromotyrosine-collagen" composites with specific elastomeric and bioactive properties for biomedical applications of 3-dimentional biomaterials.

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